CROSSLINKED HYALURONATE GELS, THEIR USE AND METHOD FOR PRODUCING THEM

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Method of preparing crosslinked gels of hyaluronic acid or derivatives of hyaluronic acid by reaction with a phosphorus-containing reagent, especially a phosphorus(V) acid derivative. Also, gels prepared by way of this method and their use as a slow-release depot for in vivo administration of hyaluronate or pharmaceutical compounds.

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(54) Title: CROSSLINKED HYALURONATE GELS, THEIR USE AND METHOD FOR PRODUCING THEM

(57) Abstract

Method of preparing crosslinked gels of hyaluronic acid or derivatives of hyaluronic acid by reaction with a phosphorus-containing reagent, especially a phosphorus(V) acid derivative. Also, gels prepared by way of this method and their use as a slow-release depot for in vivo administration of hyaluronate or pharmaceutical compounds.

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Crosslinked hyaluronate gels, their use and method for producing them

The present invention relates to a crosslinked hyaluronic acid derivative in which the crosslinking has been achieved by means of reaction with a phosphorus-containing reagent, especially a derivative of an acid of phosphorus(V). The invention moreover also relates to methods for producing such a product as well as its use as a slow release depot for administration of hyaluronic acid or a medicament, incorporated in the gel.

Hyaluronic acid is a high molecular weight polysaccharide, highly viscous in character and consisting of a disaccharide repeating unit of N-acetylglucosamine and glucuronic acid. It occurs naturally in the body of humans and animals, for instance in synovial fluid, vitreous humor and pericardial fluid. In all species, the structure of hyaluronic acid is the same whereas its molecular weight may vary within wide ranges. Because of its bioresorbability and absence of toxicologic and immunologic effects hyaluronic acid has been found to be useful in medical contexts, e.g for the treatment of articular disorders adversely affecting articular motility, and as a surgical aid in connection with eye surgery and for preventing post-operative adhesions. In such cases hyaluronic acid has been employed in the form of a viscous aqueous solution. However, in many cases the duration is too short and mechanical stabilization too weak so that the desired therapeutic effect is not attained.

Improvements in these respects have been obtained by means of chemically crosslinking the hyaluronic acid to form insoluble gels. The preparation of such gels and their use as vitreous humor substitutes and in treating retinal detachment are described in for instance U.S. patent 4716154. Controllably degradable gels, to be used in the first place as adhesion-preventing materials, are described in PCT

application WO86/00912. A feature which these hyaluronic acid gels have in common is that non-endogenous structures which are "alien" to the body are introduced into the material by way of the crosslinking procedure. This circumstance detracts from the efficacy of the basic concept of using an endogenous substance like hyaluronic acid as the matrix for the crosslinking, because the new material will actually contain structures that are "alien" to the body. As a result, the endogenous, non-toxic and non-immunogenic hyaluronic acid may undergo a change such that the crosslinked material is recognized as being "alien" - with consequential immunological and inflammatory reactions.

We have now surprisingly found a novel biologically degradable crosslinked hyaluronic acid gel derivative which is produced by means of reacting the hyaluronic acid with a phosphorus-containing reagent, especially a phosphorus(V) acid derivative, and which contains endogenous crosslinks, viz., phosphate esters. Phosphate esters occur ubiquitously in vivo. As examples may here be mentioned phospholipids, DNA and RNA.

In many other respects, too, the process for the production of the phosphate-crosslinked hyaluronate gels and their properties are superior to the manufacturing processes and properties of prior art crosslinked hyaluronic acid materials. The crosslinking reaction time is very short, and the substances require a minimum of purification because the reactive crosslinking reagents are rapidly hydrolyzed. The gels are degradable biologically, and the degradation time is variable within wide limits. In contrast to prior art crosslinked hyaluronate gels the present gels are completely re-swellable after complete desiccation.

Phosphate crosslinking of polysaccharides is a known method, primarily for the treatment of starch (see for example

Koch H et al., Stärke 34 (1982) 16). However, the derivatization of starch is a treatment of an insoluble material in a heterogeneous system. Phosphate crosslinking of hyaluronic acid too may be carried out heterogeneously on solid material, for instance in pyridine. But for obtaining a more reproducibly swelling gel material the reaction preferably chosen is one where hyaluronic acid is treated in a dissolved state with a crosslinking reagent. This treatment may be performed in an organic solvent in which the hyaluronic acid has been solubilized, e.g. by way of salt formation with a lipophilic cation. Surprisingly, however, we have found that clear, transparent gels are obtained if the reaction is carried out in an aqueous solution of hyaluronate. Carrying out the reaction in aqueous solution is preferable from a handling and purification point of view.

Crosslinking reagents employed are derivatives of phosphorus(V) acid, in particular halides, oxyhalides or anhydrides thereof. Examples of such crosslinking reagents are phosphorus pentachloride, phosphoryl chloride (phosphorus oxychloride) or the corresponding bromide or iodide, phosphorus pentoxide and trimetaphosphates.

The reaction is carried out in an alkaline medium. The coupling and the hydrolysis of the phosphorus acid derivatives result in the release of relatively large amounts of acid. Both the phosphate esters formed and the hyaluronic acid matrix are sensitive to acidic degradation. It is very important, therefore, that enough base is present already from the very start of the reaction, since it is not possible to make any additions to the viscous gelling system. At the same time, however, the pH of the initial solution must not be too high because the hyaluronic acid is sensitive to alkaline degradation. This means, in the case of crosslinking in an aqueous solution, that the pH should be one between 9 and 14 (but note that pH values in the upper part of this range can be used only if the alkaline hyaluronate solution

is prepared in situ, with cooling) and that the system has a sufficient buffer capacity. Bases that may be employed will thus be metal hydroxides such as sodium and potassium hydroxides. But already at low concentrations of these hydroxides a high initial pH will be obtained, while at the same time the buffer capacity for acid neutralization will be low. For this reason other bases, of better buffer capacity, should be employed: for example nitrogen bases like alkylamines, especially those that are sterically hindered such as triethylamine, tributylamine and methylmorpholine. However, a preferred preparation for crosslinking in aqueous solution employs basic metal phosphates like trisodium phosphate or tripotassium phosphate. In the work-up procedure of these gels the by-products obtained will only be biologically tolerable phosphate salts, there being thus no need to further proceed to purification steps for removing rests of potentially toxic alkalis. If the reaction is carried out in an anhydrous medium it is also possible to employ bases that are weaker than those mentioned above, for example pyridine.

If the crosslinking reaction is carried out homogeneously in a solution of hyaluronic acid the concentration thereof may vary within a wide range of concentrations. A practically useful concentration range is 2-15 % (by weight) of hyaluronic acid in the reaction mixture. Already concentrations as low as 1 % will give rise to gel formation, but these gels are of a very liquescent consistency and not very worthwhile for technical purposes. There is no theoretical upper limit for the hyaluronic acid concentration to be employed in the crosslinking operation. A practical upper limit, however, is set due to the circumstance that already at moderate concentrations high molecular weight hyaluronic acid will form solutions which are very difficult to work with. Therefore, in the case of a hyaluronic acid having a molecular weight of say about 10^6 the concentration should not exceed about 10 %.

The molecular weight of the hyaluronic acid employed for the crosslinking may vary within a wide range from some thousands to several millions, for example from 20,000 to $5x10^6$ depending on the concentration thereof and on the amount of crosslinking agent. However, a preferred molecular weight range is one between about 100,000 and $4x10^6$.

In addition to hyaluronic acid or hyaluronates such as e.g. the sodium salt, other derivatives of hyaluronic acid may be crosslinked in accordance with this method, such as for instance a partially sulfated hyaluronic acid or esterified hyaluronic acid (see EP 265116). This of course applies also to hyaluronic acid that has been subjected to some other minor chemical modification such as described in e.g. US 4713448.

The amount of crosslinking agent, too, may vary within a wide range depending on the molecular weight and concentration of the hyaluronic acid. In a preferred embodiment with an aqueous solution of hyaluronic acid and phosphoryl chloride as the crosslinking agent the amount of the latter may vary from 10 to 500 % by weight based on the hyaluronic acid.

The crosslinking reaction may be carried out at room temperature or at a somewhat elevated temperature. However, the reaction at these temperatures is very fast; gel formation will occur already after a few minutes' reaction time at room temperature. For better control of the reaction one will therefor choose a lower temperature, for example within the range of from 0 to 10°C.

It should be noted, however, that in a preferred reaction system where hyaluronate is crosslinked in an aqueous solution most of the crosslinking reagents are not completely soluble; instead these reagents and the aqueous phase will form a two-phase system. It is important, therefore, that the contact area between the crosslinker phase and the

hyaluronic aqueous phase be made as large as possible. We have found that the crosslinking is favored by the rheological properties of hyaluronic acid because stable suspensions or emulsions of the crosslinking reagent will form very easily. The stability of the two-phase system will also be promoted by lowered temperatures.

The gel material, after having been swollen and finely divided in a physiologically acceptable buffer, will be readily injectable. The gel can be heat-sterilized, for instance by autoclaving. Also, in addition to injectable crushed gel preparations other preparations in the form of shaped materials may be produced, like for instance films, tubes etc.

In contrast to what is the case with epoxy-crosslinked hyaluronate gels (Laurent T.C. et al. in Acta Chem. Scand. 18 (1964), 274-275) the present gels are capable of complete re-swelling after having been desiccated, for example by freeze-drying. From a manufacturing point of view this is a considerable advantage, for by storing a dry stable intermediate product one will avoid such degradation as would occur if the gel were stored while being swollen in a buffer having a pH greater than 7. Also, it is easy to alter the concentration and swelling medium of the final gel composition.

The phosphorus content in dried gels will vary from some hundredth percent up to one percent. As regards gels crosslinked in a homogeneous aqueous solution, the solids content in a completely swollen gel in aqueous solution varies between 0.1 and 10 %. On the other hand the degree of swelling will be much lower in gels crosslinked in a non-homogeneous system with an incompletely dissolved hyaluronic acid. Typically in a film thus produced the solids content will be 30 %.

The gels have a maximum of stability at pH 5.75 but are degradable at biological pH (7.3). But as compared to the carboxylate-crosslinked hyaluronate gels - which, too, are degradable and which are described in patent application WO86/00912 - the phosphate-crosslinked gels possess much greater stability. It is thus possible by means of said phophate-crosslinking to obtain hyaluronate gels having considerably longer and more variable degradation times as compared to older types of gels. Gels may now be produced with degradation times of from about one day to a couple of months. The patent application EP272300 also describes a method of extending the degradation time of hyaluronate gels by means of a combination of carboxylate ester and ether crosslinking. Those gels, however, cannot be prepared in an injectable form.

According to one aspect of the invention gels are produced which contain also other components, e.g. pharmaceuticals, this being achieved by adding the desired component to the gel either before or after the crosslinking thereof, to thus obtain for instance a slow-release effect.

In another aspect of the invention the gels are used for slow release of soluble hyaluronate in vivo. Beneficial effects of local administration of hyaluronic acid, for instance in joints, are well known from the literature. One of the problems encountered so far is that the hyaluronate, in spite of its high viscosity, is removed too quickly out of the administration area, by diffusion. The degradation products of the present gels are, as earlier mentioned, hyaluronic acid and harmless phosphates, making these gels, when implanted, excellent depots for slow-release of hyaluronic acid.

The invention thus relates to a method for crosslinking hyaluronic acid by means of subjecting a solution of hyaluronic acid or a derivative thereof to treatment with a phosphorus-containing reagent, especially a phosphorus(V)

acid derivative. The processes contemplated here are in the first place those where an aqueous solution of the hyaluronic acid or a derivative thereof is reacted in alkaline conditions, preferably pH 9-14, with a phosphorus(V) acid derivative. Preferred phosphorus(V) acid derivatives are at present considered to be halides, oxyhalides or anhydrides, for example phosphorus pentachloride, phosphoryl chloride (phosphorus oxychloride) or the corresponding bromide or iodide, phosphorus pentoxide and trimetaphosphates.

Furthermore, the invention comprises hyaluronic acid gels containing phosphate ester crosslinks produced as stated above, and the use of such gels as preparations for the administration of medicines and as slow-release depots for administration of hyaluronic acid.

A number of examples will be set forth below for the purpose of illustrating the invention, without, however, limiting the scope thereof in any way.

Unless otherwise stated a high molecular weight sodium hyaluronate (M_W $3x10^6$) has been employed for preparing the gels.

The gels have been swelled, washed and autoclaved in isotonic Sörensen buffer pH 5.75 (100 ml Na_2HPO_4 (9.470 g/l), 900 ml $NaH_2PO_4.H_2O$ (9.208 g/l) and 5200 mg NaCl per liter of solution).

The degradation times of the gels have been measured by incubation at 37°C in phosphate-buffered saline pH 7.3, complete dissolution of the gel being checked by means of filtration.

Example 1

500 mg of sodium hyaluronate were weighed into a centrifuge tube of glass. 8.3 ml of saturated trisodium phosphate (Na_3PO_4) solution were added. The polysaccharide was allowed to swell in the phosphate solution overnight in a refrigerator.

The sample was stirred with a glass rod until complete dissolution of the hyaluronate had taken place. The 6 % hyaluronate solution thus prepared has a pH of about 12.8 %.

The solution was cooled in an ice bath to about +1°C. 167 µl of phosphoryl chloride ($POCl_3$) were added with vigorous stirring. After a few minutes of stirring the solution gelled. The sample was stirred during five minutes all in all, whereupon it was centrifuged until a homogeneous clear gel was obtained.

After a further ten minutes' reaction time the resultant gel, which had a neutral pH, was cut into thin slices. The slices were transferred to one liter of Sörensen buffer. The gel was washed for two days with shaking, the buffer being replaced three times during this period. The yield of swollen gel was 35 ml.

The gel was crushed by being forced through a fine-meshed steel wire net and was filled into syringes which were then autoclaved. The soft, slightly cohesive gel could easily be injected through a fine injection needle.

The non-autoclaved gel had a degradation time of 5 weeks whereas the autoclaved gel was degraded within 3-4 weeks. The phosphorus content of the dialyzed dried gel amounted to 0.081 %. The solids content of the swollen gel was 1.4 %.

Example 2

300 mg of sodium hyaluronate were swelled in 15 ml of water overnight in a refrigerator. By means of stirring the hyaluronate was made to dissolve completely so as to form a 2 % solution. To this were added 3.75 g of solid sodium phosphate ($Na_3PO_4.12H_2O$). After the salt had dissolved the solution was cooled in an ice bath, whereupon crosslinking was carried out with 400 µl $POCl_3$ as according to Ex. 1.

The gel thus formed was dialyzed against dist. water for two days; then the material was crushed and freeze-dried. The phosphorus content was 0.10 %. The dried gel was allowed to swell in Sörensen buffer for three days, and was then autoclaved. The gel thus obtained was cohesive, homogeneous, entirely clear, fairly mobile. The degradation time of the autoclaved gel amounted to 4-5 days.

Example 3

The experiment of Ex. 2 was repeated, but this time a 3 % solution was prepared with 300 mg of hyaluronate in 10 ml of water, with 2.5 g of $Na_3PO_4x12H_2O$ as the base. 250 µl of $POCl_3$ were employed as the crosslinking agent.

In this case the swollen gel had a solids content of 3.6 %. The degradation time of the autoclaved gel was 10 days. The phosphorus content of the dried gel was 0.085 %.

Example 4

400 mg of sodium hyaluronate were dissolved in 3.3 ml of water to thus form a very thick 12 % solution. The sample was cooled to about +10°C whereupon 1 ml of triethylamine (which is water-soluble below +18°C) was added, with mixing. The pH of the solution was about 13.5. The sample was subjected to further cooling, down to +1°C, and was cross-linked with 183 µl of $POCl_3$.

A rigid gel was obtained having a phosphorus content of 0.12 %. In autoclaved state, the gel had a degradation time of 4 weeks.

Example 5

5 ml of 6 % hyaluronate solution in water were mixed with 1 ml of 4-methylmorpholine; the pH of the solution was about 11.2. The solution was crosslinked with 200 µl of $POCl_3$ as stated above.

A somewhat liquescent gel was obtained. The gel was autoclaved on having been swelled in Sörensen buffer of pH 5.0. The non-autoclaved gel had a degradation time of between 15 and 19 days while after autoclaving the degradation time was 10 days.

Example 6

In this example, crosslinking was tested in aqueous solutions with various different concentrations of sodium hydroxide as the base.

To 2.75 g of cooled 10 % hyaluronate solution were added varying amounts of sodium hydroxide solution whereupon crosslinking was effected with a predetermined amount of crosslinker, 100 µl $POCl_3$.

a. 100 µl 5 M NaOH. After the $POCl_3$ addition the solution became rapidly very acidic (pH about 2). Only a very small amount of water-insoluble gel was obtained.

b. 250 µl 5 M NaOH. This too resulted rapidly in the formation of a very acidic reaction solution. The gel obtained had a very liquescent consistency and could not be autoclaved without being degraded.

c. 500 µl 5 M NaOH. After crosslinking, a gel of rather firm consistency was obtained. In its fully swollen condition it had a hyaluronate content of 0.6 %. However, autoclaving resulted in considerable degradation of the gel. The autoclaved sample had a degradation time of 4 days.

d. 1000 µl 5 M NaOH. The hyaluronate was rapidly degraded by the alkaline liquor so as to form a solution of low viscosity. Addition of the crosslinker did not result in gel formation.

These examples clearly show that adequate buffer control is required in the crosslinking system.

Example 7

300 mg of sodium hyaluronate were dissolved in 3 ml of saturated Na_3PO_4 solution so as to form a 10 % solution which was then crosslinked with 25 µl of $POCl_3$. A rigid gel was obtained which had a degradation time amounting to 14 days.

Example 8

80 mg of acid-degraded sodium hyaluronate having a molecular weight of 100,000 were dissolved in 1 ml of saturated Na_3PO_4 solution. To this was added a further 0.2 g of solid $Na_3PO_4.12H_2O$. The solution was crosslinked with 75 µl $POCl_3$.

An opalescent brittle gel having a degradation time of two weeks was obtained.

Example 9

300 mg of sodium hyaluronate were dissolved in 5 ml of saturated Na_3PO_4 solution. To the cooled solution 200 mg of

phosphorus pentachloride (PCl_5) were added in small aliquots and with vigorous stirring. The phosphorus pentachloride reacted more rapidly and more violently than phosphoryl chloride. A somewhat opalescent gel was obtained which had a degradation time of two weeks.

Example 10

200 mg of tetrabutyl ammonium hyaluronate were dissolved in 2 ml of dimethylformamide. This solution was admixed with 1 ml of triethylamine, whereupon the solution was cooled and 100 µl of $POCl_3$ were added. Gel formation was almost instantaneous. The gel that had been formed consisted of white flakes showing very little tendency of swelling in water. The gel had a hyaluronate content amounting to 30 %.

Example 11

100 mg of tetrabutyl ammonium hyaluronate were dissolved in 2 ml dimethylformamide. 200 µl of triethylamine were added, and the solution was cooled and admixed with 200 mg of diphosphorus pentoxide (P_2O_5). Almost immediately a white gel precipitate was formed, having the same properties as the gel prepared according to Ex. 10.

Example 12

100 mg of sodium hyaluronate were evaporated with 3x10 ml dry pyridine. The substance was suspended in 10 ml of pyridine, whereupon the suspension was cooled and 400 µl of $POCl_3$ were added. The solution was shaken for 15 minutes. The hyaluronate thus treated, which is insoluble in water, will be liable to irregular swelling to thus form a mixture of hard white portions and clear gel portions. The hardest crosslinked white gel portions are not degradable at a physiological pH but will dissolve when subjected to alkaline treatment. The phosphorus content in the dialyzed dried gel was 0.77 %.

Example 13

Dialyzed salt-free sodium hyaluronate was dried in a petri dish so as to form a clear, planar film. The film was allowed to swell for some seconds in a cooled mixture of one part of water and nine parts of triethylamine. The swollen film was treated with phosphoryl chloride, either by being dipped into it for some seconds or by being maintained in phosphoryl chloride vapor during about one minute. In both cases a clear, water-insoluble crosslinked film was obtained; in its swollen state it had a solids content of about 30 %.

CLAIMS

1. Method of preparing gels of crosslinked hyaluronic acid or derivatives thereof, characterized in that a solution of the hyaluronic acied or a derivative thereof is crosslinked by reaction with a phosphorus-containing reagent.

2. Method according to claim 1, characterized in that the reagent is a phosphorus(V) acid derivative.

3. Method according to claim 2, characterized in that the phosphorus(V) acid derivative is a halide, an oxyhalide or an anhydride.

4. Method according to claim 3, characterized in that the phosphorus(V) acid derivative is phosphorus pentachloride, phosphoryl chloride or phosphorus pentoxide.

5. Gel of crosslinked hyaluronic acide or derivative thereof, characterized by having been prepared by means of reacting the hyaluronic acid or a derivative thereof with a phosphorus(V) acid derivative.

6. Gel of crosslinked hyaluronic acid or derivative thereof, characterized in that the crosslinks are phosphate ester bridges.

7. Method for the administration of soluble hyaluronic acid in vivo, characterized in that a gel of phosphate ester crosslinked hyaluronic acid or a derivative thereof is implanted as a slow-release depot.

8. Method for the administration of a pharmaceutical in vivo, characterized in that a gel of phosphate ester crosslinked hyaluronic acid or a derivative thereof in which the pharmaceutical is incorporated, is implanted as a slow-release depot.

9. Use of a gel of phosphate ester crosslinked hyaluronic acid or a derivative thereof for in vivo administration of soluble hyaluronic acid.

10. Use of a gel of phosphate ester crosslinked hyaluronic acid or a derivative thereof in which a pharmaceutical has been incorporated as a slow-release depot for in vivo administration of said pharmaceutical compound.

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 90/00077

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 08 B 37/08, A 61 K 47/36, A 61 K 31/725

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 08 B; A 61 K;

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	US, A, 4605691 (BALAZS ET AL) 12 August 1986, see column 1, line 55 - line 62; column 4, line 53 - line 56; column 5, line 4 - line 13 --	1-10
A	US, A, 3422088 (JOHN V. TUSCHHOFF ET AL) 14 January 1969, see column 4, line 10 - line 15; abstract --	1-3
A	US,A, 3555009 (SHIGEO SUZUKI ET AL) 12 January 1971, see abstract --	1

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27th April 1990	1990 -05- 04
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Agneta Österman Wallin

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 7-8, because they relate to subject matter not required to be searched by this Authority, namely:

Methods for treatment of the human or animal body by therapy (PCT rule 39 (iv)).

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the the claims. It is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additonal fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional seach fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/SE 90/00077

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 90-03-30
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A- 4605691	86-08-12	AU-B-	569157	88-01-21
		AU-B-	572419	88-05-05
		AU-D-	4304585	86-06-12
		AU-D-	7217387	87-08-27
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		DE-A-	3520008	86-06-19
		FR-A-B-	2574414	86-06-13
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		JP-A-	61138601	86-06-26
		SE-A-	8503486	86-06-07
		US-A-	4582865	86-04-15
		US-A-	4636524	87-01-13
US-A- 3422088	69-01-14	BE-A-	684964	67-02-02
		DE-A-	1567393	70-04-16
		FR-A-	1536186	00-00-00
		GB-A-	1082335	00-00-00
		NL-A-	6610899	67-02-03
US-A- 3555009	71-01-12	BE-A-	700254	67-12-01
		DE-A-	1567368	71-09-02
		NL-A-	6704368	67-12-22